

## Research report

# Effects of age and diet on the heavy particle-induced disruption of operant responding produced by a ground-based model for exposure to cosmic rays

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Accepted 11 December 2004

Available online 2 February 2005

## Abstract

On missions to other planets, astronauts will be exposed to galactic cosmic rays which are composed of heavy particles (such as <sup>56</sup>Fe) and protons. Exposure to these particles can affect the ability of rats to perform a variety of tasks, indicating that there is the possibility that the performance capabilities of astronauts may be affected. Previous research has shown that diets containing blueberry or strawberry extract can ameliorate the deficits produced by irradiation using a ground-based analog for exposure to cosmic rays. Rats were placed on diets containing 2% blueberry or strawberry extract for 2 months prior to exposure to 1.5 Gy of 1 GeV/n <sup>56</sup>Fe particles. There were no effects on performance of any group of animals when tested on an ascending fixed-ratio operant task 6 months following exposure. When tested 12 months after exposure, the performance of the radiated animals given blueberry extract did not differ from the radiated animals fed the control diet. Both groups performed significantly poorer than the non-irradiated controls. There were no differences between the non-irradiated animals fed control diet and the radiated animals fed the strawberry diet and their performance was significantly better than of the radiated rats fed the blueberry or control diets. The results indicate that diets containing strawberry extract may provide a significant level of radiation protection on exploratory class missions.

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*Theme:* Neural basis of behavior*Topic:* Aging*Keywords:* <sup>56</sup>Fe particles; Oxidative stress; Antioxidant diets; Conditioning

## 1. Introduction

On exploratory class missions, such as a mission to Mars, astronauts will be exposed to doses and types of radiation that differ from those experienced in Low Earth Orbit, where the International Space Station and Space Shuttle operate [1]. This radiation is composed primarily of protons and of particles of high energy and charge (HZE particles) such as <sup>56</sup>Fe [2,19,32]. Previous research, using a ground-

based model for exposure to cosmic rays, has shown that exposure to <sup>56</sup>Fe particles produces changes in dopaminergic system function and in behaviors that depend upon the integrity of this system. HZE particle-induced deficits have been observed in motor behavior (upper body strength) [13], amphetamine-induced taste aversion learning (CTA) [24,25], spatial learning using the Morris water maze [30], and operant responding [23,27].

The neurochemical and behavioral deficits observed following exposure to <sup>56</sup>Fe particles are also observed in aged animals, including decreases in potassium-stimulated dopamine release, in upper body strength and in spatial learning and memory [9,11,12,31]. As such, it has been pro-

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posed that exposure to HZE particles produces “accelerated aging” [14,18]. A common factor underlying the neurochemical and behavioral effects of both aging and exposure to ionizing radiation may be oxidative stress. The cumulative effects of the production of reactive oxygen species and oxidative stress are one of the factors associated with the aging process [7,10]. Exposure to ionizing radiation [3,28] and to HZE particles [6] also produces oxidative stress. Research by Joseph et al. [4,16,17,34] has shown that maintaining rats on antioxidant diets containing strawberry or blueberry extract can ameliorate the neurochemical and behavioral changes that are characteristic of the aged organism. Specifically, maintaining senescent rats on diets containing strawberry or blueberry extract for 2 months produced increases in oxotremorine-enhanced DA release from striatal slices when compared to senescent animals given the control diets [16]. In addition to the improvement in dopaminergic function, there were improvements in motor behavior and in spatial learning and memory [4,17,34]. Similarly, rats maintained on these diets for 2 months prior to exposure to 1.5 Gy of  $^{56}\text{Fe}$  particles fail to show an HZE particle-induced deficit in the acquisition of an amphetamine-induced CTA [26] which is mediated by the dopaminergic system.

These results suggest that it might be possible to utilize diets containing antioxidant phytochemicals to provide protection against the range of behavioral disruptions produced by exposure to HZE particles. To evaluate this possibility, the present experiment examined the effects of antioxidant diets on the disruption of an ascending fixed-ratio operant response following exposure to low doses of  $^{56}\text{Fe}$  particles. This behavior was selected because it is dependent upon the integrity of dopaminergic system and because the effectiveness of dopaminergic lesions changes as a function of the age of the organism [21,20,29]. Unlike intact rats, rats with 6-hydroxydopamine-induced lesions of the dopaminergic system fail to show a corresponding increase in response rate as the reinforcement ratio increases [21,20,29]. In addition, partial lesions of the dopaminergic system that do not affect performance in younger rats do affect the performance of older rats [20]. Because operant responding can be considered a model system for all forms of complex learning, the effects of antioxidant diets on the performance of this task may have implications for astronaut performance on the range of complex tasks required for the successful completion of their mission.

## 2. Materials and methods

### 2.1. Subjects

The subjects were 60 male Sprague–Dawley rats weighing 175–200 g at the start of the experiment. They were housed in AAALAC-accredited animal facilities at Brookhaven National Laboratory (BNL) where they were main-

tained on a 12:12 light/dark cycle. Food and water were continuously available except as required by the experimental protocol. The animal protocols were approved by the IACUCs of both BNL and University of Maryland Baltimore County (UMBC). Two months prior to irradiation, rats were placed on diets containing either 2% blueberry or strawberry extract or a control diet. Twenty rats were fed each diet. For irradiation, half the rats in each diet condition were irradiated while the remaining rats served as non-irradiated controls.

### 2.2. Diets

The procedures used to prepare the extracts have been described previously [17,35]. In brief, strawberries and blueberries were homogenized in water (1:1 or 2:1 w/v, respectively) for 3 min. The homogenate was centrifuged at  $13,000 \times g$  for 15 min at 4 °C. The supernatant was collected, lyophilized and the freeze dried extracts were shipped to Harlan Teklad (Madison, WI) where it was combined with the control diet, which was a modification of the NIH-31 diet. The amount of corn in the control diet was adjusted to compensate for the addition of the strawberry or blueberry extracts.

### 2.3. Radiation

The Alternating Gradient Synchrotron at BNL was used to irradiate the rats with  $^{56}\text{Fe}$  particles. During exposure to the particles, the rats were restrained using a well-ventilated plastic tube which was placed perpendicular to the beam and positioned so that the head of the rat was in the center of the beam. Because the beam was approximately 7 cm in diameter, the neck and shoulders of the rat may have received some radiation. An X-ray film was taken to confirm the location of the rat within the beam. The rats were exposed laterally to 1.5 Gy of 1 GeV/n  $^{56}\text{Fe}$  particles at a nominal dose rate of 1.0–1.5 Gy/min. Control rats were not exposed to the beam. The details of the beam and dosimetry have been provided by Zeitlin et al. [36].

### 2.4. Operant training and testing

Prior to being shipped to UMBC, the rats were tested for the effects of diet on the acquisition of an amphetamine- or lithium chloride-induced CTA [26]. The pharmacokinetics of both compounds indicates that they would have been rapidly cleared from the system and would not have affected performance 2 months or more after use. At UMBC, the rats were placed on a diet of standard laboratory rat chow (Purina 5100). They were given 6–8 weeks to recover from the effects of shipping before beginning acquisition training on the operant task. The first run using the ascending fixed-ratio reinforcement schedule was begun 6 months following exposure to  $^{56}\text{Fe}$  particles; the second run was begun 12 months following irradiation. Prior to each run, the rats were weighed and placed on a food deprivation schedule to

reduce their weight to 85–90% of their pre-deprivation weight. Body weight was maintained by giving the rats access to reduced amounts of food. Throughout the training and testing periods, the rats were weighed daily and the food obtained by responding in the operant chamber was supplemented by experimenter-provided chow to maintain their weight at the reduced level.

The initial acquisition of the bar pressing task was accomplished using an autoshaping procedure. The rats were placed in the operant chambers (Coulbourn Instruments, Allentown, PA) for 12-h blocks of time separated by 12 h in their home cages. During this time, all bar-presses were rewarded by the delivery of a 45 mg food pellet. Using this protocol, only one to three sessions were needed for all rats to learn the response. Once all rats had learned to bar-press to obtain food, fixed-ratio (FR) training was begun. On consecutive days, the rats were placed on FR-1, FR-1, FR-5, FR-10, and FR-20 reinforcement schedules. At the conclusion of the FR training, the rats were maintained in the home cages with food and water continuously available until the start of testing.

The first test session was run 6 months following irradiation. The second test session was run 12 months following irradiation. Both the first and second runs followed the same sequence. First, the rats were retrained to respond on a fixed-ratio schedule described above and then tested on an ascending fixed-ratio schedule starting with FR-1 and proceeding on consecutive days to FR-5, FR-10, FR-15, FR-20, FR-25, FR-30, and FR-35.

Although the initial sample size was 10 rats/group, there was a decrease in size as a function of age and tumor development. This was particularly acute in the irradiated animals fed the control diet, such that only two of these rats were alive 12 months following exposure [15]. The loss of subjects in the radiated rats fed the control diet was primarily due to the effects of radiation on tumor development. The rats were euthanized with an overdose of sodium pentobarbital when the tumors reached a diameter of  $\approx 3$ –4 cm or when open sores developed. Sample sizes for each condition are provided in the figure legends.

### 2.5. Data analysis

Initial data analysis was performed using a three-way ANOVA with one repeated factor, reinforcement schedule, for each of the two runs independently. Where a significant triple interaction was obtained, a series of post hoc comparisons between the six treatment (3 diet  $\times$  2 radiation conditions) groups were performed using Fisher's LSD (protected *t* tests) to compensate for the use of multiple *t* tests.

## 3. Results

The results for the first run (6 months after irradiation) are summarized in Fig. 1. The data for the non-irradiated and

irradiated rats have been separated to enhance clarity. Fig. 1A presents the responses of all non-irradiated animals. Fig. 1B presents the responses of the irradiated animals compared to the non-irradiated rats fed the control diet. A three-way ANOVA indicated that only the main effect for reinforcement schedule was significant ( $F[7,350] = 33.18$ ,  $P < 0.001$ ). Neither the main effect for radiation (radiated/non-irradiated) nor diet (blueberry/strawberry/control) was significant. In addition, none of the three interactions was significant.

For the second run (12 months after irradiation), both the main effect for reinforcement schedule ( $F[2,259] = 32.74$ ,  $P < 0.001$ ) and the triple interaction (diet  $\times$  radiation condition  $\times$  reinforcement schedule) ( $F[14,259] = 1.86$ ,  $P < 0.05$ ) were significant. None of the other comparisons achieved significance. To analyze the significant triple interaction, post hoc comparisons between the six treatment (3 diet  $\times$  2 radiation conditions) groups were run using Fisher's LSD (protected *t* tests) to compensate for the use of multiple *t* tests. Starting at a reinforcement schedule of FR-20, the non-irradiated rats maintained on the control diet and the radiated animals maintained on the strawberry diet responded at a significantly higher rate than the radiated rats maintained on the control or blueberry diets (Fig. 2B). The differences in response rate between the non-irradiated rats maintained on the control diet and the radiated rats maintained on the strawberry diet were not significant. Similarly, the differences in responding between the radiated rats maintained on the control or blueberry diet were not significant.

With regard to the non-irradiated rats, the analysis using Fisher's LSD indicated that the rats maintained on both the blueberry and strawberry diets showed significantly lower response rates than the rats maintained on the control diet at most reinforcement schedules greater than FR-15 (Fig. 2A). The response of the non-irradiated rats on both the blueberry and strawberry diets was not significantly different than the response rates of radiated animals. With the exception of the FR-30 ratio, there were no significant differences in response rate between the rats maintained on the strawberry or blueberry diet.

Because there was a significant diet  $\times$  radiation condition  $\times$  ratio interaction for the second run but not for the first run, a second set of ANOVAs was run comparing the responses of the radiated and control rats on the two runs which were separated by 6 months. There were no significant differences in the responses of the non-irradiated rats across the two runs. In contrast, the radiated rats showed a significant ratio  $\times$  run ( $F[7,168] = 2.26$ ,  $P < 0.05$ ) and diet  $\times$  run  $\times$  reinforcement schedule ( $F[14,168] = 2.81$ ,  $P < 0.001$ ) indicating that the pattern of responding for the radiated, but the non-irradiated rats varied across the two runs.

## 4. Discussion

Exposing rats to low doses of  $^{56}\text{Fe}$  particles disrupts the functioning of the dopaminergic system and the behaviors

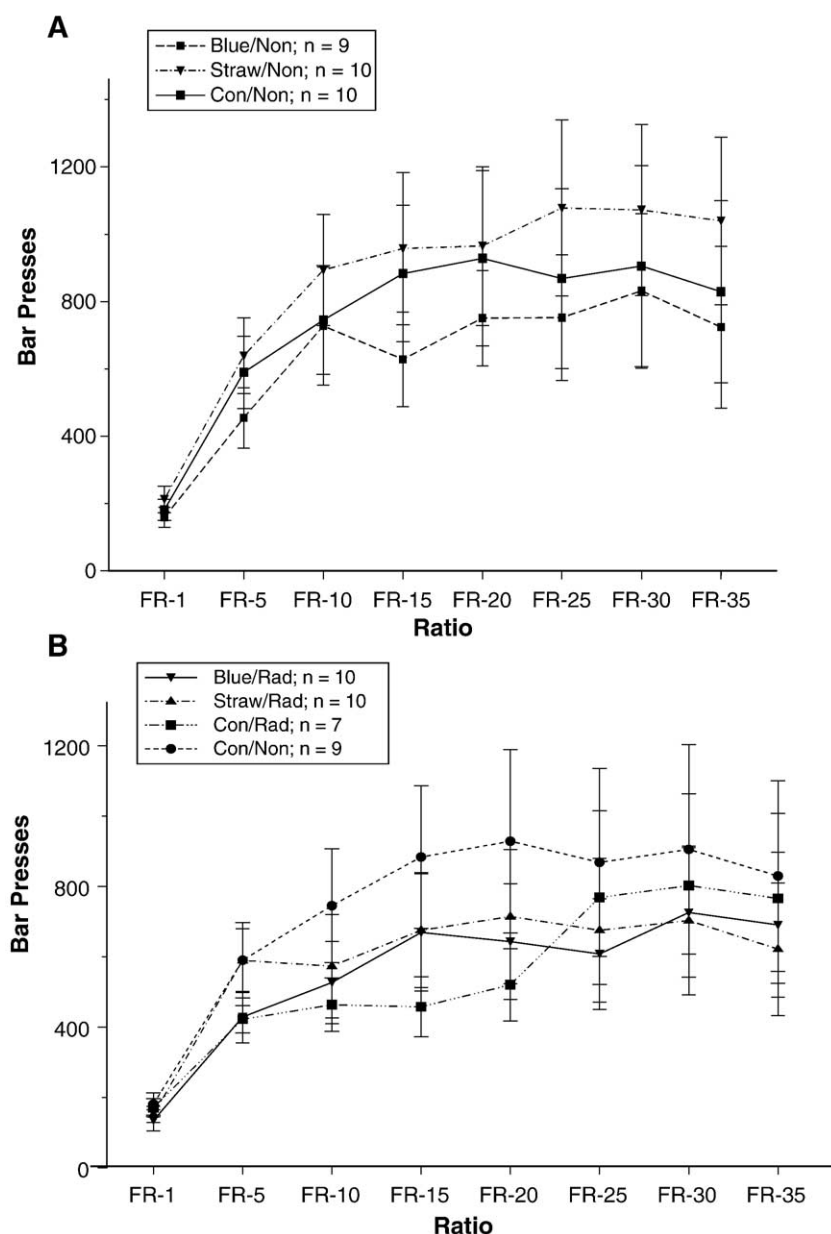


Fig. 1. Effects of exposure to  $^{56}\text{Fe}$  particles on operant responding on an ascending fixed-ratio task 6 months after exposure. The sample size is given in the figure legends. The error bars indicate the standard error of the mean. (A) Non-irradiated rats. (B) Radiated rats. The performance of the non-irradiated rats fed a control diet is included for comparison.

which are dependent upon the integrity of that system [13,23–25,27,30]. Deficits similar to those produced by exposure to HZE particles are observed in aged rats [9,11,12,31]. It has been proposed, therefore, that exposure to these particles produces “accelerated aging” in young animals [14,18]. This suggests that similar mechanisms may be responsible for the behavioral deficits observed in aging and following exposure to HZE particles. Current theories of aging emphasize the role of oxidative stress in the aging process [7,10] and exposing young rats to ionizing radiation [3,28] and to  $^{56}\text{Fe}$  particles [6] produces oxidative stress.

Consistent with the hypothesized role of oxidative stress in aging, Joseph et al. [4,16,17,34] have reported that

maintaining rats on antioxidant diets ameliorates the neurochemical and behavioral deficits that characterize the aging process. This research has suggested that senescent rats supplemented for 2 months on strawberry or blueberry diets similar to those utilized here showed increases in oxotremorine-enhanced DA release from striatal slices when compared to senescent animals given the control diets [16]. Concordant with the improvement in dopaminergic function, there are also improvements in the behaviors that depend upon the integrity of the dopaminergic system, including motor behavior, measured by the ability for a rat to maintain its grip on a wire, and spatial learning and memory, measured by performance on the Morris water maze [4,16,17,34].

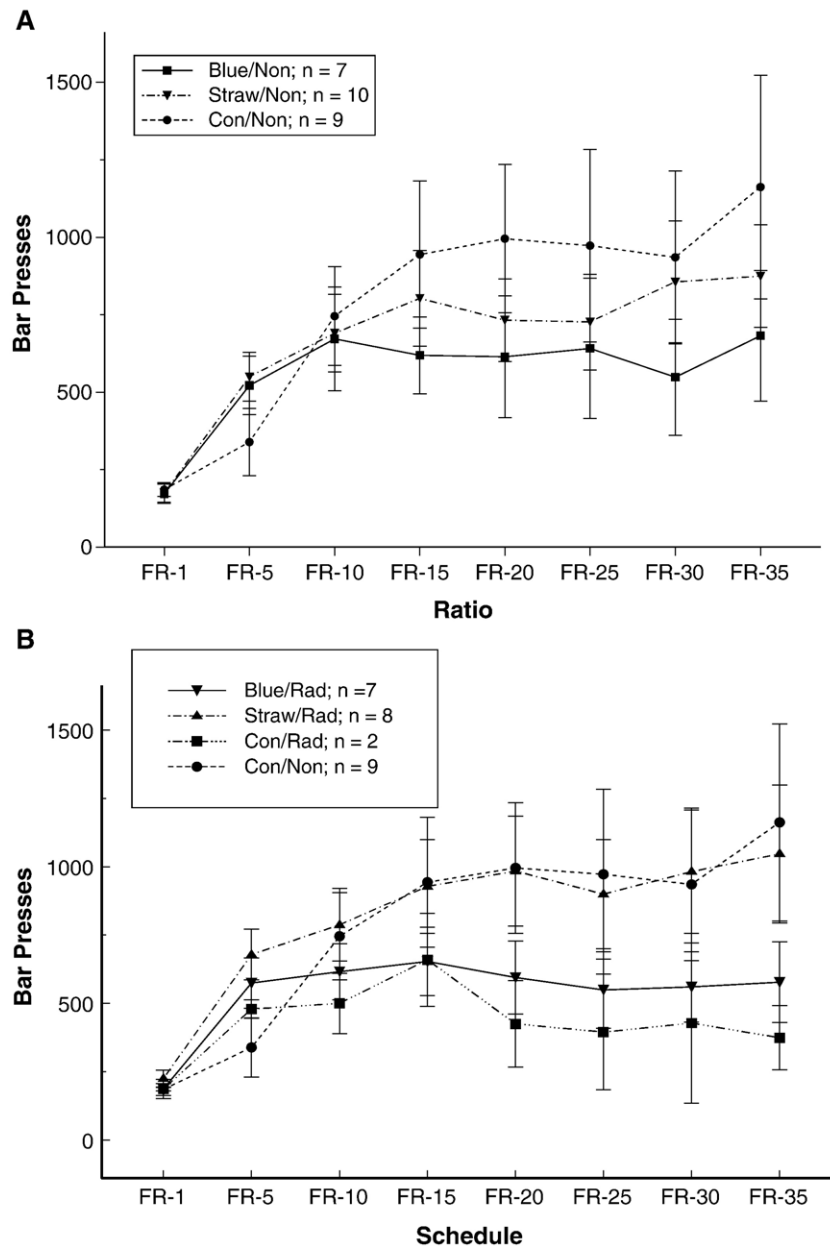


Fig. 2. Effects of exposure to  $^{56}\text{Fe}$  particles on operant responding on an ascending fixed-ratio task 12 months after exposure. The sample size is given in the figure legends. The error bars indicate the standard error of the mean. (A) Non-irradiated rats. (B) Radiated rats. The performance of the non-irradiated rats fed a control diet is included for comparison.

With regard to the effects of antioxidant diets on HZE-induced changes in performance, previous research has shown that rats maintained on diets containing 2% strawberry or blueberry extract fail to show the  $^{56}\text{Fe}$ -induced disruption of CTA learning produced by injection of amphetamine [26]. Overall, the results of the present experiment showing that maintaining rats on an antioxidant diet can prevent the disruption responding on an ascending fixed-ratio operant task produced by exposure to heavy particles are consistent with past research on the role of oxidative stress in mediating the effects of HZE particle irradiation on behavior [6].

When rats are tested within 6 months following exposure to  $^{56}\text{Fe}$  particles, there is no effect on operant performance. These results are consistent with previous research in showing that testing rats on an operant task 5–6 months following exposure to 1.5 Gy of 1 GeV/n  $^{56}\text{Fe}$  particles does not affect operant performance. Rather, the threshold for an effect on operant performance at that time is 2.0 Gy [23,27]. Because there are no significant differences in operant performance 6 months following irradiation, there are no differences in responding as a function of diet. At this time, all rats showed equivalent increases in response rate as a function of increases in the reinforcement schedule.



When tested 12 months after irradiation, there were significant differences between the treatment groups as a function of radiation condition and diet. The performance of the radiated rats that had been maintained on the diet containing 2% strawberry extract prior to irradiation was identical to that of the non-irradiated rats maintained on the control diet. In contrast, there were no differences in the performance of the radiated rats maintained on either the control diet or the diet containing 2% blueberry extract. There were no differences in performance at the lower reinforcement schedules as a function of either radiation condition or diet. However, as the reinforcement ratio became greater than FR-15, the radiated animals fed the control or blueberry diets showed significantly reduced performance compared to the non-irradiated rats fed the control diet or the radiated rats fed the strawberry diet. This observation is consistent with the effects of intrastriatal injection of 6-hydroxydopamine on operant responding, in which deficits in fixed-ratio performance are observed only at the higher ratios [8,20,21,29].

The factors that are responsible for the greater effectiveness of the strawberry diet compared to the blueberry diet are not clear. The difference in the effectiveness of these diets may be unique to this particular task because both diets are equally effective in preventing the  $^{56}\text{Fe}$  particle-induced disruption of amphetamine-induced taste aversion learning [26]. Because both blueberry and strawberry diets similar to those utilized here produced increases in oxotremorine-enhanced DA release from striatal [16] tissue, it is unlikely that the difference in the effectiveness of the two diets on operant performance in irradiated rats is due to differences in blood–brain barrier permeability. Rather, the effects of these diets on the striatum and the observation that performance on an ascending fixed-ratio task can be disrupted by intrastriatal injection of 6-OHDA [8,20,21,29] suggest that these diets must exert their effects on central dopaminergic systems.

In terms of their free radical scavenging capacity, blueberries have been reported to have a greater oxygen radical absorbance capacity than strawberries [22,33]. To the extent that the disruption of performance on the ascending fixed-ratio operant task depends upon the generation of radiation-induced oxidative stress or inflammation, the greater capacity of blueberries to scavenge free radicals would suggest that they would be more effective in ameliorating the effects exposure to  $^{56}\text{Fe}$  particles on behavior. The fact that blueberries were not more effective than strawberries suggests that factors in addition to the ability to scavenge free radicals must play a role in ameliorating specific effects produced by exposure to HZE particles. The polyphenols in fruit are complex and the specific components have not been tested to determine the active compound(s) responsible for their radioprotective actions.

The observation during the second run that the non-irradiated animals maintained on the blueberry or strawberry

diets showed poorer performance than did the non-irradiated rats fed the control diet was not predicted. In general, the performance decrement of the non-irradiated rats fed the strawberry and blueberry diets paralleled that of the radiated animals in that there were no differences in response rate at the lower reinforcement schedules, but significant differences in responding were observed at ratios greater than FR-15. Because the rats were taken off the diet 12 months prior to the run, the performance decrement could not have resulted from the current effects of the diets on the neural mechanisms that mediate this response. It may be that maintaining the rats on these diets for 2 months prior to irradiation had some unanticipated effects on striatal neurochemical systems. While similar deficits in performance resulting from the use of antioxidant diets have not been reported in studies using aged animals, it is possible that differences in the nature of the oxidative stress account for the observation of deficits in the present experiment. In studies of aging, oxidative stress occurs chronically at a low level, whereas following irradiation there is the acute production of a higher level of oxidative stress. If this is a factor in the performance decrement produced by berry diets, then maintaining the rats on the diet for a shorter period of time (e.g. 2 weeks) should provide a degree of radiation protection in the absence of a diet-produced performance decrement in the non-irradiated rats. Alternatively, it may be that the F-344 rats typically used in studies of aging [4,16,17,34] respond differently to the diet than the S-D rats used in the present study. Nonetheless, it should be noted that the decreased performance of the non-irradiated rats fed blueberry extract cannot account for the failure of the blueberry diet to protect against the HZE particle-induced performance decrement because an equivalent decrease was observed with the non-irradiated rats fed strawberry extract.

In summary, the present results show that maintaining rats on a diet containing 2% strawberry extract can prevent the disruption of responding on an ascending fixed-ratio operant task produced by exposure to a ground-based analog of cosmic rays. These results suggest that dietary manipulations can be used to provide protection to astronauts on exploratory class missions outside the protection provided by the magnetosphere. Because operant responding can be considered a model system for complex learning, the present results may have implications for astronaut performance on the range of complex tasks required for the successful completion of their mission. The effects of exposure to HZE particles might become manifest during the projected 3-year duration of a manned mission to Mars, interfering with the ability of astronauts to successfully complete mission requirements, or they might become manifest many years after completion of the mission, interacting with the changes due to normal aging to produce neurodegenerative diseases such as Parkinson's. While this possibility remains speculative at the present time, it may be noted that there are data which suggest that

the occurrence of cataracts is greater in retired astronauts than in the population at large [5], suggesting that the effects of low dose/low dose rate radiation in space might become manifest years after exposure. Nonetheless, the present results suggest that the use of antioxidant diets during missions to other planets may provide long-term protection for astronauts exceeding the duration of the mission.

## Acknowledgments

This research was supported by Grants NAG9-1190 and NAG9-1529 from the National Aeronautics and Space Administration.

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